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Effect of moderate hydrostatic pressures on the enzymatic activity and bioactive composition of pineapple by-products

Diana I. Santos[1] | Carlos A. Pinto[2] | Luiz C. Corrêa-Filho[1] | Jorge A. Saraiva[2] | António A. Vicente[3] | Margarida Moldão-Martins[1]

[1]LEAF, Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Lisbon, Portugal

[2]QOPNA & LAQV-REQUIMTE, Química Orgânica, Produtos Naturais e Agroalimentares, Departamento de Química, Universidade de Aveiro, Campus Universitário de Santiago, Aveiro, Portugal

[3]CEB, Centre of Biological Engineering, University of Minho, Braga, Portugal

Correspondence
Margarida Moldão-Martins, LEAF, Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisbon, Portugal.
Email: mmoldao@isa.ulisboa.pt

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Abstract

The application of abiotic stresses by moderate hydrostatic pressures (MHP) is still underdeveloped. Abiotic stresses allow activating the enzymatic complexes inducing the synthesis of de novo bioactive compounds. Pineapple by-products are rich in bromelain and bioactive compounds that can be enhanced through abiotic stresses. The aim of this study was to evaluate the effect of MHP on the enzymatic activity of pineapple by-products. Pineapple by-products were submitted to MHP (50–400 MPa between 1 and 15 min) according to a central composite factorial design matrix. Samples were stored at 5 ± 1°C for 24 hr, to allow enzymatic activity to occur. Enzymatic and antioxidant activities and total phenolic compounds (TPC) were quantified. MHP promoted a 262% increase in the phenylalanine ammonia-lyase activity and 36% increase in TPC, in shell samples. In core the activity of bromelain increased 350%. These results pinpoint the potential to increase the value of pineapple by-products by enhancing the amounts of bioactive compounds through MHP application.

Practical application

Abiotic stresses can enhance enzyme activity, inducing the synthesis of bioactive compounds in living tissues. Hydrostatic pressure is an innovative nonthermal process that can be used to stabilize or increase enzymes' activity present in by-products generated in the minimally processed fruit and vegetables industry. Moderate hydrostatic pressure (MHP) act as abiotic stress inducing de novo phenols synthesis and enhancing bromelain activity. After treatment, enriched material could be stabilized and then blended with foods and beverages to improve nutraceutical properties and help in the prevention and treatment of chronic diseases. The study demonstrates that MHP (150–250 MPa) applied to the pineapple core and pineapple shell produce a phenolic and bromelain rich product.

1 | INTRODUCTION

Bioactive compounds' levels in fruits and vegetables can be enhanced by the application of controlled postharvest abiotic stresses, to induce de novo synthesis of active compounds. Different stresses can activate specific enzymes involved in the synthesis of the corresponding compound (Cisneros-Zevallos, 2003) and an increase in the activity of enzymes related with the biosynthesis and accumulation of secondary metabolites can be associated with late response of plants to abiotic stresses (Jacobo-Velázquez, González-Agüero, & Cisneros-Zevallos, 2015).

Moderate hydrostatic pressure (MHP) can be used to stabilize or increase enzymes' activity and this enzyme activity enhancement is an effective response parameter with great potential for application in

enzyme catalysis (Eisenmenger & Reyes-De-Corcuera, 2009). The behavior of enzyme activity is variable: depending on the hydrostatic pressure applied and on the food matrix, the inactivation or activation of the enzyme may occur. Some studies reported increase of enzymatic activity in fruits and vegetables using MHP (Chakraborty, Kaushik, Rao, & Mishra, 2014). The increase in enzyme activity after pressurization may occur due to the reversibility of the enzyme's conformation or reorganization of its active sites, modification of the substrate or of the medium properties, and/or displacement of the equilibrium towards the release of inhibitors from enzymes when enzyme-inhibitor complexes are formed (Eisenmenger & Reyes-De-Corcuera, 2009). Once the enzyme is unfolded by pressure, it may become more sensitive to the substrate (Chakraborty, Kaushik, et al., 2014; Eisenmenger & Reyes-De-Corcuera, 2009).

Enzymatic reactions in plant tissues can be enhanced by favoring the contact between the enzyme and the substrate, for example, caused by the disruption of tissue that occurs during stresses, particularly mechanical stresses such as application of hydrostatic pressure. Hydrostatic pressure may also alter the conformation of macromolecular substrates, increasing or decreasing the easiness of the catalytic action of the enzyme upon them, with this consequently affecting the enzyme-substrate interaction and, ultimately, enzyme activity (Cano & Ancos, 2005).

Pineapple is a fruit that contains large amounts of proteolytic enzymes, namely stem bromelain (EC 3.4.22.32) and fruit bromelain (EC 3.4.22.33) that are homologous cysteine proteases (Raimbault, Zuily-Fodil, Soler, Mora, & Cruz de Carvalho, 2013) which are absorbed by the body without losing proteolytic activity and without creating significant side effects. Bromelain has numerous advantages in the digestive and cardiovascular systems. Bromelain has anticancer properties, promotes apoptosis (cell death), relieves osteoarthritis, diarrhea and various cardiovascular disorders, and has also therapeutic benefits, such as for the treatment of angina pectoris, bronchitis, sinusitis, surgical trauma and thrombophlebitis, wound debridement, and absorption of drugs, principally antibiotics (Pavan, Jain, Shraddha, & Kumar, 2012).

Phenylalanine ammonia lyase (PAL; EC 4.3.1.5) is very important in biosynthesis of phenolic compounds and can be induced by stress conditions. In the phenylpropanoid pathway, PAL is the first enzyme and plays an essential role in the biosynthesis of phenolic compounds in plants (Chen et al., 2006; Tomás-Barberán & Espín, 2001). PAL is responsible for the catalysis of nonoxidative deamination of L-phenylalanine forming trans-cinnamic acid and a free ammonium ion. Plant cells were shown to increase the synthesis of PAL in response to hydrostatic pressure treatments stress, this resulting in an increase of the synthesis of polyphenols (Terefe, Buckow, & Versteeg, 2014).

MHP may also increase or decrease the catalytic action of other important enzymes in plant tissues, such as pectin methyl esterase (PME; EC 3.1.1.11), polyphenol oxidase (PPO; E.C. 1.14.18.1), peroxidase (POD; EC 1.11.1.7) are usually very resistant to hydrostatic pressures (HP).

The by-products from fresh-cut fruit and vegetable industries are still physiologically active living tissues, able to synthetize compounds, and thus they can be used as biofactories of secondary metabolites with pharmaceutical and nutraceutical applications (Surjadinata & Cisneros-Zevallos, 2012). The abiotic stresses can activate some enzymatic antioxidant systems of the fresh fruit and, consequently, enhance the antioxidant capacity (Cisneros-Zevallos, 2003). This study intends to evaluate the influence of moderate hydrostatic pressure treatments on the increase of enzymatic activity in pineapple by-products and the corresponding effect on the accumulation of bioactive compounds by those tissues.

2 | MATERIAL AND METHODS

2.1 | Reagents and solutions

Folin–Ciocalteu reagent and cysteine ($C_3H_7NO_2S$) were purchased from Panreac AppliChem (Germany). 2,2-difenil-1-picrilhidrazil (DPPH; $C_{18}H_{12}N_5O_6$), 2,4,6-tripyridyl-s-triazine (TPTZ; $C_{18}H_{12}N_6$), poly (vinylpolypyrrolidone) $(C_6H_9NO)_n$, triton X-100 (t-Oct-C_6H_4-[OCH_2CH_2] xOH, x = 9–10), casein from bovine milk, glycine ($C_2H_5NO_2$), β-mercaptoetanol (C_2H_6OS), pectin from citrus peel (galacturonic acid ≥74%), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS; $C_{18}H_{18}N_4O_6S_4$) were acquired from Sigma–Aldrich (Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Acrós Organics (Belgium). Catechol ($C_6H_6O_2$), tyrosine ($C_9H_{11}NO_3$), L-phenylalanine ($C_9H_{11}NO_2$) and cresol red ($C_{21}H_{17}NaO_5S$) were purchased from Alfa Aesar (United Kingdom).

2.2 | Sample preparation

Pineapple by-products (*Ananas comosus* L.) were provided by company Campotec S. A. located in Torres Vedras, west center of Portugal. The shell (9.27 ± 0.60 °Brix) and core (10.17 ± 0.67 °Brix) were stored under refrigeration (4 ± 1°C) ~18 hr prior to packaging and abiotic stresses application. The by-products of the pineapple were cut in specific dimensions: core cylinders (~52.5 × 30 mm) and the shell (~110 × 40 mm). Subsequently, the by-products were packaged (~40 g) in PA/PE-90 (Alempack-Embalagens Flexíveis, Elvas, Portugal) that were vacuum sealed (85% of vacuum). The samples were prepared in triplicate for each treatment of the experimental design.

2.3 | Moderate hydrostatic pressures treatment

The packaged by-products were processed in a pilot-scale high hydrostatic pressure equipment (Hiperbaric 55, Burgos, Spain) with a 55 L vessel, according to a central composite factorial matrix (Tables 1 and 2), with the pressure conditions varying between 50 and 400 MPa and the processing times between 1 and 15 min. Samples were stored at ±5°C for 24 hr and then were frozen at −80°C until the analyzes were performed.

TABLE 1 Experimental design with coded and decoded values of independent variables (pressure and time) and responses for pineapple shell: bromelain activity, phenylalanine ammonia-lyase activity (PAL), polyphenoloxidase activity (PPO), pectin methylesterase activity (PME), total phenolic compounds (TPC), and antioxidant activity by methods DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric Reducing Antioxidant Power) and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid))

Run	Coded factors		Decoded factors		Bromelain (μmol tyrosine. $min^{-1}.g^{-1}$ dry matter)	PAL (μmol t-cinnamic acid. $h^{-1}.g^{-1}$ dry matter)	PPO ($U.g^{-1}$ dry matter)	PME ($U.g^{-1}$ dry matter)	TPC (mg CAE.g^{-1} dry matter)	DPPH (μmol Trolox. g^{-1} dry matter)	FRAP (μmol Trolox. g^{-1} dry matter)	ABTS (μmol Trolox. g^{-1} dry matter)
	P	t	P (MPa)	t (min)								
12 (C)	0	0	225	8	27.44 ± 4.45	388.48 ± 19.12	130.50 ± 3.72	7.71 ± 0.34	96.54 ± 0.70	36.39 ± 1.08	40.00 ± 0.52	39.62 ± 0.87
11 (C)	0	0	225	8	28.27 ± 1.70	367.52 ± 5.51	130.13 ± 4.60	8.91 ± 0.21	95.79 ± 0.91	37.20 ± 1.37	40.36 ± 0.44	40.17 ± 0.68
9 (C)	0	0	225	8	25.16 ± 1.03	360.91 ± 47.51	137.57 ± 2.59	8.40 ± 0.52	95.43 ± 0.64	37.97 ± 0.36	41.19 ± 0.75	39.05 ± 0.63
3	1	−1	350	3	22.57 ± 0.48	116.99 ± 13.78	116.86 ± 0.10	9.82 ± 0.91	82.61 ± 2.44	36.86 ± 0.15	35.90 ± 0.12	37.29 ± 0.20
10 (C)	0	0	225	8	28.61 ± 6.72	384.90 ± 10.74	135.07 ± 2.63	9.00 ± 0.06	95.57 ± 0.64	36.20 ± 1.02	41.11 ± 0.84	39.43 ± 1.17
5	−α	0	50	8	17.61 ± 1.94	110.54 ± 5.34	155.71 ± 0.53	8.10 ± 0.90	79.17 ± 1.54	34.16 ± 0.85	35.10 ± 0.38	34.98 ± 0.49
2	−1	1	100	13	21.82 ± 2.29	194.65 ± 16.97	81.52 ± 0.73	10.34 ± 0.53	75.02 ± 1.98	28.26 ± 1.27	28.15 ± 0.33	33.80 ± 0.59
8	0	α	225	15	14.72 ± 1.01	107.19 ±11.14	81.24 ± 0.86	7.92 ± 0.53	71.08 ± 0.65	22.94 ± 0.25	27.31 ± 1.18	29.20 ± 0.55
6	α	0	400	8	19.35 ± 3.87	115.94 ± 23.29	90.25 ± 0.10	11.76 ± 0.90	76.11 ± 3.48	20.53 ± 0.37	25.15 ± 0.43	27.36 ± 1.12
4	1	1	350	13	12.21 ± 2.71	134.19 ± 13.49	108.14 ± 0.10	9.34 ± 0.52	74.96 ± 2.05	23.08 ± 0.11	22.97 ± 0.80	35.86 ± 0.16
1	−1	−1	100	3	17.51 ± 3.22	112.44 ± 8.43	130.66 ± 0.99	10.52 ± 0.53	86.42 ± 2.02	31.11 ± 0.79	36.97 ± 1.71	46.40 ± 1.33
7	0	−α	225	1	21.72 ± 1.58	145.62 ± 23.77	140.88 ± 0.16	9.67 ± 1.05	73.79 ± 2.80	33.42 ± 0.33	38.65 ± 0.26	48.22 ± 1.02

2.4 | Experimental design

Response surface methodology (RSM) was used to find the most favorable experimental conditions. The experiments were carried out following a central composite rotatable design (CCRD), as a function of pressure (50–400 MPa) and time (1–15 min). A total of 12 experiments were carried out (Table 1—Shell and Table 2—core): four factorial design points (± 1), four axial points (± 1.414), and four central points (0). The repetition of the central point was used to determine the experimental error, which was assumed to be constant throughout the experimental domain. The experiments were performed randomly in order to avoid systematic errors.

The experimental data were statistically analyzed through a stepwise multiple regression analysis using StatisticaTM v.8 Software (StatSoft Inc., 2007), which was fitted to a second-order polynomial equation to predict each dependent variable [Enzymatic activity (Bromelain, PAL, PPO, PME), TPC, antioxidant activity (DPPH, FRAP, ABTS)] (Y). The three-dimensional response surface designs as a function of independent variables [pressure (X_1; MPa) and time (X_2; min)], b_0 is the interception and b_i, b_j, b_{ij} (i,j = 1,2) are the linear, quadratic, and interaction coefficients, respectively that are described by the second order polynomial models, using decoded variables, as follows (Equation (1)).

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \quad (1)$$

The adequacy of the model to the experimental data was confirmed by the analysis of variance (ANOVA) and coefficient of determination (R^2) and adjusted R^2 (Adj-R^2) (Montgomery, 2017).

The desirability function was applied to experimental results to optimize multiple responses in RSM for shell samples: bromelain activity and phenylalanine ammonia-lyase (PAL) activity. This function is highly useful when optimizing complex systems (Corrêa-Filho, Lourenço, Duarte, Moldão-Martins, & Alves, 2019).

2.5 | Analytical methods

The preparation of the pineapple extract for the enzymatic activities is described together with the quantification method. All experimental results were determined from extracts of the samples. Each extract was analyzed in triplicate and the average was used for each condition.

2.5.1 | Bromelain activity

The bromelain assay was determined according to Chakraborty, Rao, and Mishra (2014) with some modifications. Two grams of pineapple sample was mixed with 20 ml extraction solution (5 mmol/L ethylenediamine-tetra-acetic acid [EDTA] and 25 mmol/L cysteine prepared in 0.1 mol/L sodium phosphate buffer [pH 8]) in an ice bath for 2 min using an Ultra-Turrax (Ika Labortechnik T25 basic) at 8000 rpm. The mixture was centrifuged (Hermle Labortechnik Z 383 K) at 8000 rpm

TABLE 2 Experimental design with coded and decoded values of independent variables (pressure and time) and responses for pineapple core: bromelain activity, phenylalanine ammonia-lyase activity (PAL), polyphenoloxidase activity (PPO), pectin methylesterase activity (PME), total phenolic compounds (TPC), and antioxidant activity by methods DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric Reducing Antioxidant Power) and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid))

Run	Coded factors		Decoded factors		Bromelain (μmol tyrosine. $min^{-1}.g^{-1}$ dry matter)	PAL (μmol t-cinnamic acid. $h^{-1}.g^{-1}$ dry matter)	PPO ($U.g^{-1}$ dry matter)	PME ($U.g^{-1}$ dry matter)	TPC (mg $CAE.g^{-1}$ dry matter)	DPPH (μmol $Trolox.g^{-1}$ dry matter)	FRAP (μmol $Trolox.g^{-1}$ dry matter)	ABTS (μmol $Trolox.g^{-1}$ dry matter)
	P	t	P (MPa)	t (min)								
12 (C)	0	0	225	8	15.91 ± 0.45	117.81 ± 5.80	101.30 ± 1.25	7.99 ± 0.65	78.74 ± 0.99	40.49 ± 0.19	30.23 ± 0.62	37.79 ± 0.58
11 (C)	0	0	225	8	16.97 ± 0.18	117.73 ± 6.36	104.78 ± 2.73	8.80 ± 0.14	77.93 ± 1.81	41.81 ± 0.21	30.96 ± 2.18	36.85 ± 0.64
9 (C)	0	0	225	8	17.71 ± 1.45	117.10 ± 5.40	101.00 ± 3.12	8.06 ± 0.69	77.66 ± 1.08	41.49 ± 0.97	30.85 ± 1.17	37.35 ± 0.31
3	1	−1	350	3	3.93 ± 0.32	114.05 ± 3.13	85.92 ± 3.17	8.45 ± 0.64	71.16 ± 1.24	32.71 ± 0.37	25.78 ± 0.65	36.48 ± 0.38
10 (C)	0	0	225	8	17.01 ± 1.33	117.89 ± 5.43	99.44 ± 0.10	8.93 ± 0.62	77.43 ± 1.02	39.24 ± 0.58	29.70 ± 0.81	36.51 ± 0.51
5	−α	0	50	8	13.59 ± 0.86	124.00 ± 3.11	123.42 ± 0.10	8.71 ± 0.94	80.53 ± 1.05	43.61 ± 1.10	19.16 ± 0.69	51.60 ± 0.71
2	−1	1	100	13	8.11 ± 0.23	121.45 ± 5.35	75.80 ± 3.20	8.48 ± 0.68	76.70 ± 1.79	33.38 ± 0.30	26.31 ± 0.34	29.91 ± 0.95
8	0	α	225	15	11.92 ± 0.81	112.19 ± 5.42	78.44 ± 3.09	9.57 ± 0.69	74.83 ± 0.85	33.96 ± 0.89	23.50 ± 0.32	30.83 ± 0.17
6	α	0	400	8	8.41 ± 0.96	124.14 ± 3.07	86.34 ± 3.18	10.15 ± 0.63	79.95 ± 1.30	41.25 ± 0.44	33.24 ± 0.99	37.60 ± 0.76
4	1	1	350	13	13.83 ± 1.08	128.95 ± 5.44	84.96 ± 3.13	10.57 ± 0.81	74.47 ± 1.54	38.05 ± 0.67	24.94 ± 0.43	42.42 ± 0.21
1	−1	−1	100	3	12.09 ± 0.52	116.59 ± 3.15	102.44 ± 0.10	11.60 ± 0.63	80.23 ± 1.27	39.45 ± 1.04	33.73 ± 0.75	46.24 ± 0.54
7	0	−α	225	1	7.58 ± 0.31	113.51 ± 3.07	109.21 ± 3.10	8.82 ± 0.18	81.62 ± 0.78	42.32 ± 0.46	36.39 ± 1.33	50.92 ± 0.69

for 20 min at 4°C and the supernatant was filtered (Whatman no.1) and used as crude enzyme extract.

The reaction blend consisted of 50 μl enzyme extract, 1,150 μl of 1% (wt/vol) casein solution in 0.1 mol/L glycine and 25 mmol/L cysteine. The mixture was incubated in a shaking water bath (10 min at 37°C) and the reaction was subsequently stopped by adding 1.8 ml of 5% (wt/vol) trichloroacetic acid (TCA). The assay mixture was filtrated (0.45 μm) and the absorbance was taken at 280 nm (UNICAM UV/Vis Spectrometer). In the blank sample, TCA was added before the addition of casein substrate. Bromelain activity was calculated using a standard curve established with tyrosine (0–50 mg/L), and expressed as the amount of tyrosine on a dry weight basis and reported in the units $\mu mol.min^{-1}.g^{-1}$ (dry weight basis).

2.5.2 | Phenylalanine ammonia-lyase activity

The phenylalanine ammonia-lyase (PAL) activity determination was performed as described in Alegria, Gonçalves, Moldão-Martins, Cisneros-Zevallos, and Abreu (2016) with few modifications. The pineapple sample (2 g) was added to 2 g polyvinylpolypyrrolidone and homogenized with 20 ml of 50 mmol/L borate buffer (pH 8.5) containing β-mercaptoethanol (400 μl/L). This mixture was kept into an ice-bath and homogenized in an Ultra-Turrax for 2 min at 8000 rpm. Homogenates were centrifuged at 8000 *g* for 20 min at 4°C and the supernatant collected, filtered (Whatman no.1) and used as crude enzyme extract.

The PAL reaction mixture was performed by addition 2000 μl of the borate buffer, 600 μl of L-phenylalanine (100 mmol/L) substrate solution and 400 μl of crude enzyme extract. The blank reactions were prepared as described using nanopure water as a substitute L-phenylalanine (100 mmol/L) substrate solution. The samples were read before and after 1 hr of incubation in bath water (40°C) in a spectrophotometer at 290 nm after being blanked with borate buffer. The PAL activity was expressed as the amount of synthesized t-cinnamic acid on a dry weight basis and reported in the units $\mu mol.h^{-1}.g^{-1}$ (dry weight basis).

2.5.3 | Polyphenol oxidase activity

The extraction of polyphenol oxidase (PPO) followed a modified method of Zhou, Dahler, Underhill, and Wills (2003). The sample material (2 g) was homogenized with 20 ml 0.1 mol/L sodium phosphate buffer at pH 6.5 at 4°C, 10% polyvinylpolypyrrolidone (wt/wt) and Triton X-100 for 2 min in an Ultra-Turrax at low speed (8,000 rpm) in an ice-bath to avoid excess heating and to prevent protein denaturation. The homogenate was then centrifuged at 8000 rpm for 20 min at 4°C and filtered (Whatman no.1). The supernatant was used for PPO activity assay.

PPO activity was assayed spectrophotometrically by a modified method based on Babu, Rastogi, and Raghavarao (2008). The assay mixture consisted in 2.5 ml of substrate solution (50 mmol/L catechol

in 0.1 mol/L phosphate buffer, pH 6.5) and enzyme extract to a final reaction volume of 3.0 ml. The rate of catechol oxidation was followed at 420 nm for 1 min. An enzyme activity unit was defined as the amount of the enzyme that causes an increase of 1.0 in absorbance per minute per milliliter ($\Delta Abs.min^{-1}.g^{-1}$) (dry weight basis).

2.5.4 | Pectin methylesterase activity

The pectin methylesterase (PME) activity was assayed according to Chakraborty, Rao, and Mishra (2014) and Pinheiro, Silva, Alegria, Abreu, and Gonçalves (2012), with some modifications. PME from the pineapple samples (2 g) was extracted using 20 ml 1.5 mol/L sodium chloride solution and was mixed with homogenizer during 2 min at low speed (8,000 rpm) in an ice-bath. The mixture was centrifuged at 8000 *g* for 20 min at 4°C, and the supernatant was filtered (Whatman no.1) and adjusted to the pH 8.8 with NaOH 1 and 0.1 mol/L. The reaction mixture contained 2,600 μl pectin from citrus fruits (0.5%), 150 μl cresol red (0.01%) in sodium phosphate buffer at 0.003 mol/L and pH 8.8, and 250 μl enzyme extract. The absorbance was measured at 573 nm during 1 min. The definition used for 1 unit (U) of enzyme activity was the amount of enzyme that produced a change in absorbance of 1.0 per min per g of sample ($\Delta Abs.min^{-1}.g^{-1}$) (dry weight basis), under assay conditions.

2.5.5 | Pineapple extract preparation for TPC and antioxidant activity

The pineapple extract preparation was evaluated following the procedure of Heredia and Cisneros-Zevallos (2009) and Swain and Hillis (1959), with some modifications. The extracts preparation was carried out in a ratio of 1:10 (wt:vol) of three pineapple by-products independent sample and methanol (100%) followed by Ultra-Turrax homogenizer at 8000 rpm for 2 min. The homogenates were incubated overnight (12–24 hr) at 4°C. The extracts were obtained by centrifugation at 8000 rpm for 20 min (4°C), and the supernatants were stored at 4°C, protected from light until analysis.

2.5.6 | TPC

The TPC were determined according Heredia and Cisneros-Zevallos (2009), and Swain and Hillis (1959), with some modifications. The 150 μl extract aliquots were diluted with 2,400 μl nanopure water, followed by 150 μl of 0.25 mol/L Folin–Ciocalteu and incubated for 3 min at room temperature. The reaction was stopped by adding 300 μl of 1 mol/L Na_2CO_3 and the mixture was incubated for 2 hr protected from light. The supernatant samples were read at 725 nm absorbance. The total phenolics for each sample was determined by using a standard curve developed with equivalent chlorogenic acid (CAE) and expressed as mg CAE/g^{-1} dry weight.

2.5.7 | Antioxidant activity (DPPH assay)

The antioxidant capacity by DPPH (2,2-diphenyl-1-picrylhydrazyl) method was evaluated following the procedure of Brand-Williams, Cuvelier, and Berset (1995) with some modifications. The DPPH solution was prepared with methanol until reaching 1.1 units of absorbance at 515 nm. The sample extracts were prepared as described above. The sample (100 μl) were taken from the supernatants and then added with a 3,900 μl DPPH solution. This mixture was homogenized, and the reaction occurred for 40 min in the dark. Subsequently, the samples were read in a spectrophotometer at 515 nm. The blank was prepared with methanol and used as a control. The antioxidant activity was determined using Trolox for the standard curve (100 to 1,500 μmol/L), and the results were expressed by the Trolox Equivalent Antioxidant Capacity (TEAC [μmol/g] [dry weight basis]).

2.5.8 | Antioxidant activity (FRAP assay)

The FRAP (Ferric Reducing Antioxidant Power) assay was performed according to Benzie and Strain (1996) with some modifications. Initially the preparation of several solutions including 300 mM acetate buffer (3.1 g sodium acetate [$C_2H_3NaO_2{\cdot}3H_2O$] and 16 ml glacial acetic acid [$C_2H_4O_2$]), pH 3.6, 10 mmol/L TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mmol/L HCl, and 20 mmol/L $FeCl_3{\cdot}6H_2O$ solution. The working solution was prepared by mixing 35 ml acetate buffer 300 mmol/L, 3.5 ml TPTZ solution, and 3.5 ml $FeCl_3{\cdot}6H_2O$ solution. The reaction started with the mixture of 2.7 ml of the FRAP solution, 270 μl H_2O and 90 μl the extract samples and then warmed in water bath at 37°C for 30 min. The colored product (ferrous tripyridyltriazine complex) were read at 595 nm using water as blank. The antioxidant capacity was calculated using a standard curve established with Trolox and the results are expressed as TEAC.

2.5.9 | Antioxidant activity (ABTS-assay)

Antioxidant activity was measured using ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) method as described by Re et al. (1999) and Rufino et al. (2007) with some modifications. Two stock solutions of ABTS (7 mmol/L) and potassium persulfate (140 mmol/L) were prepared. The working solution was prepared by mixing 2 ml of ABTS solution with 35.2 μl of potassium persulfate solution and keeping it in the dark at room temperature for 12–16 hr. The ABTS solution was then diluted with methanol to obtain an absorbance of 0.700 ± 0.05 at 734 nm. The reaction was performed by mixture 2,970 μl ABTS solution with 30 μl sample aliquots, during 6 min and the absorbance at 734 nm was immediately recorded. The absorbance of the reaction samples was compared to the Trolox standard and the results were expressed in terms TEAC.

3 | RESULTS AND DISCUSSION

3.1 | General discussion

The experimental results of response variables [Enzymatic activity (Bromelain, PAL, PPO, PME), TPC, antioxidant activity (DPPH, FRAP, ABTS)] are shown in Tables 1 and 2 for pineapple shell and core, respectively. Regression analysis was performed in order to statistically evaluate the quadratic models developed. Table 3 shows the linear and quadratic effects of the independent variables, as well as their interaction, on responses for each studied dependent variable. Determination coefficients, R^2 and Adj-R^2 and significance of lack of fit are also presented. Just the models with a determination coefficient (R^2) higher than 0.75, indicating a good fit to the experimental data, are presented (Haaland, 1989).

3.2 | Bromelain activity

The quadratic models generated for bromelain activity in the pineapple shell and core were significant in fitting of the experimental data within a confidence level of 95%. High values for R^2 and Adj-R^2 (Table 3) indicated a good fit to the data. Furthermore, the adequacy of the second-order polynomial models was confirmed by the not significant lack of fit ($p > .05$).

From Table 3, linear and quadratic terms of pressure and time, as well as their interaction, were significant on the response models for bromelain activity in both pineapple shell and core samples. The effect of linear terms was positive whereas the effect of quadratic terms was negative. The interaction of the variables pressure and time had a positive effect in the bromelain activity of the pineapple core and negative effect in the activity of the bromelain in pineapple shell.

Figure 1 exhibits the prediction of bromelain activity as a function of pressure (MPa) and time (min) of the MHP treatment, based on the regression models developed for shell (a) and core (b). The figures, convex surfaces, show that bromelain tends to increase with increasing time and pressure, but at higher pressures (> 300 MPa) the enzyme activity decreases due to partial inactivation. The moderate hydrostatic pressure (200–250 MPa) and treatment time (5–10 min) promoted the increase of 134% in bromelain activity in pineapple shell and 350% in pineapple core. The optimal pressure and time conditions for the enzymatic activity of bromelain were 235.5 MPa and 6.74 min for the pineapple shell and 211 MPa and 8.7 min for the pineapple core samples.

A moderate pressure between 100 and 300 MPa has a protective effect against thermal denaturation of fruit bromelain at temperatures (30–70°C), while inactivation rate decrease with increasing pressure at a given temperature (Chakraborty, Rao, & Mishra, 2016b).

Fruit bromelain resistance in crude extract depends on its structure, and homology differs between eight diverse isoforms with a wide molecular weight range that exhibit an additional stability of this enzyme during processing with HP (Bhattacharyya, 2008; Chakraborty et al., 2016b).

The increase in bromelain activity can be explained by the formation of enzymes through different biosynthesis pathways due to the defense mechanism of plant tissues when subjected to stress, or leaching of enzymes from other cell compartments after pressurization (Orozco-Cardenas, Narvaez-Vasquez, & Ryan, 2001).

In other studies, the residual activity of bromelain in pineapple puree increased firstly and subsequently decreased (Chakraborty, Rao, & Mishra, 2016a). Enzyme activity presented the same behavior

TABLE 3 Regression coefficients of second-order polynomial equations for each decoded response variable [P - pressure (MPa) and t – time (min)]: bromelain activity, phenylalanine ammonia-lyase activity (PAL), polyphenoloxidase activity (PPO), total phenolic compounds (TPC), and antioxidant activity by methods DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric Reducing Antioxidant Power) and ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid))

Parameter	Pineapple sample	Equation	R^2	R^2 adj	Lack of fit
Bromelain activity (BRM)	Shell	$BRM = -5.613 + 0.173\ P^{*} - 0.0003\ P^{2*} + 3.847\ t^{*} - 0.183\ t^{2*} - 0.006\ Pt^{*}$	0.95	0.91	Not significant
	Core	$BRM = 6.453^{*} + 0.038\ P^{*} - 0.0002\ P^{2*} + 1.513\ t^{*} - 0.154\ t^{2*} + 0.006\ Pt^{*}$	0.97	0.95	Not significant
Phenylalanine ammonia–lyase activity (PAL)	Shell	$PAL = -390.908^{*} + 3.825\ P^{*} - 0.008\ P^{2*} + 84.169\ t^{*} - 4.823\ t^{2*} - 0.026\ Pt$	0.97	0.94	Not significant
Total phenolic compounds (TPC)	Shell	$TPC = 52.069^{*} + 0.209\ P^{*} - 0.0005\ P^{2*} + 5.871\ t^{*} - 0.424\ t^{2*} + 0.002\ Pt$	0.93	0.86	Significant
Antioxidant activity (DPPH)	Shell	$DPPH = 15.669 + 0.140\ P^{*} - 0.0003\ P^{2*} + 2.680\ t^{*} - 0.155\ t^{2*} - 0.004\ Pt$	0.86	0.74	Significant
Antioxidant activity (FRAP)	Shell	$FRAP = 21.884^{*} + 0.150\ P^{*} - 0.0004\ P^{2*} + 1.990\ t - 0.161\ t^{2*} - 0.002\ Pt$	0.97	0.95	Significant
Antioxidant activity (ABTS)	Shell	$ABTS = 50.075^{*} + 0.044\ P - 0.0002\ P^{2*} - 2.333\ t^{*} + 0.019\ t^{2} + 0.004\ Pt$	0.88	0.78	Significant

*$p < .05$.

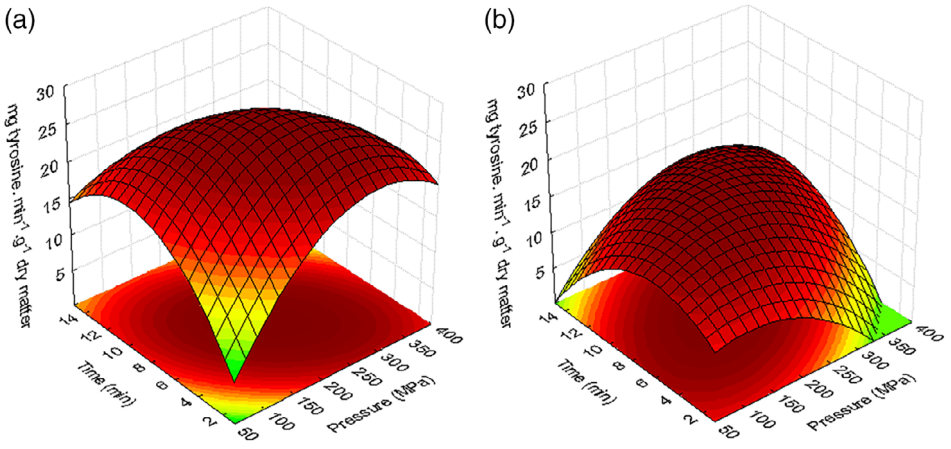

FIGURE 1 Response surfaces fitted for bromelain activity as a function of pressure (MPa) and time (min) treatment: (a) pineapple shell and (b) pineapple core

during storage time (45 days at 4°C) of avocado paste and mango pulp (Jacobo-Velázquez & Hernández-Brenes, 2010).

3.3 | PAL activity and TPC

The second-order polynomial model generated for PAL activity of the shell samples presents a good fit to the data (R^2 = 0.97 and Adj-R^2 = 0.94) and a not significant lack of fit (Table 3). The effect of linear terms was positive whereas the effect of quadratic terms was negative, as well as the effect of the interaction of the variables.

Model obtained for TPC presents a good fit to the data (R^2 = 0.93 and Adj-R^2 = 0.86) but a significant lack of fit (Table 3), so these results will be considered only as indicative of a trend. The significance of each individual, interactive, and quadratic terms towards the TPC is the same observed for the PAL activity.

The response surface presented in Figure 2 shows that the PAL activity of the shell samples showed an increase for the treatments at higher pressures (150–300 MPa) and longer dwell times (5–10 min), despite a decrease occurs for higher pressures (> 300 MPa) and very long times (> 12 min). Pineapple shell samples showed an increase in the enzymatic activity of PAL (262%) and, consequently, an increase of TPC (36%), compared with the raw material (Table 1). Moderate pressures (150–300 MPa) and time treatment (5–10 min) also seems to increase the content of TPC.

The pineapple shell presented optimum conditions of pressure and time for the enzymatic activity of PAL at 221.5 MPa and 8.14 min.

Regarding pineapple core, models obtained for PAL activity and TPC present an insufficient fit to the data (R^2 < 0.75) and a correspondingly significant lack of fit and thus are not presented. PAL activity and TPC in pineapple core increased average 15 and 14%, respectively, in relation to raw material (Table 2). In stressed samples the enzymatic activity of PAL ranged between 112.19 and 128.95 μmol t-cinnamic acid.h^{-1}.g^{-1} dry matter and TPC values ranged between 71.16–81.62 mg CAE.g^{-1} dry matter in the pineapple core samples.

PAL is an important enzyme in the phenylpropanoid pathway that has an important role in the secondary metabolism of plants,

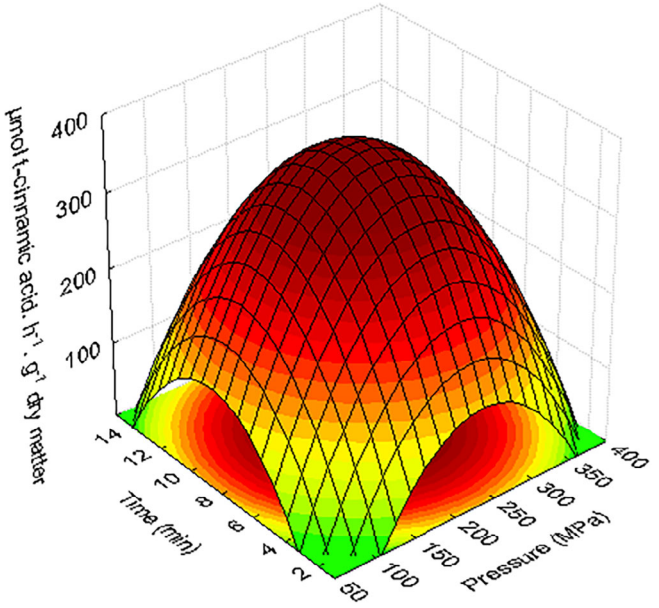

FIGURE 2 Response surfaces fitted for phenylalanine ammonia-lyase activity (PAL) as a function of pressure (MPa) and time (min) treatment in pineapple shell

and forms a diversity of phenolic compounds with structural and protection-related functions, as well as lignins, phenolic acids, stilbenes, and flavonoids (Chen et al., 2006; Solecka & Kacperska, 2003).

The increase in TPC with moderate hydrostatic pressure treatment can be explained by an increase in pressure-induced extraction in the pineapple by-product samples, similarly to that observed in pineapple puree (Chakraborty et al., 2016a). High-pressure treatments may also origin variations in enzyme conformation and enzyme substrates, which can facilitate or inhibit enzyme catalyzed reactions (Mozhaev, Lange, Kudryashova, & Balny, 1996). The rate of enzymatic reactions in the tissue of the plant may increase due to treatments with moderate pressure, since there is rupture of plant tissues and contact between enzyme and substrate (Ludikhuyze, Van Loey, Smout, & Hendrickx, 2003).

The synthesis of TPC in plant tissues is related to the enzymatic activity of PAL. The behavior of PAL activity is similar to the behavior of TPC present in fruits, since the decrease in PAL activity caused a decrease in TPC, thus demonstrating the importance of PAL in the promotion of TPC (Zarei, Zamani, Fattahi, Salami, & Mousavi, 2016).

The main phenolic compounds found to be present in pineapple were gallic acid, catechin, and epicatechin, contributing to a 40% increase in antioxidant capacity, followed by vitamin C and β-carotene. Phenolic concentration is related to the increase of PAL enzymatic activity, which is influenced by fruit ripening (Rosas et al., 2018).

3.4 | PPO and PME activity

In general, the studied variables in experimental conditions do not significantly influence the PPO and PME activities. Obtained models present an insufficient bad fit to the data ($R^2 < 0.75$) and a correspondingly significant lack of fit and thus are not presented.

PPO activity values range from 81.24 to 155.71 $U.g^{-1}$ of dry matter in pineapple shell and 75.80–123.42 $U.g^{-1}$ of dry matter in pineapple core. Although the model is not significant, the respective response surface allows observing the trend described in the literature. The use of pressures lower than 100 MPa activates some enzymes, particularly monomeric enzymes such as PPO (Buckow, Weiss, & Knorr, 2009). In the present study, hydrostatic pressures lower than 100 MPa during less than 8 min increased PPO activity and pressures higher than 300 MPa and treatment times longer than 10 min decrease the activity of this enzyme. The behavior of PPO enzymatic activity under the studied pressure and temperature conditions can be seen as an advantage, since PPO activity is associated with a negative impact on color of fruit products.

PPO showed reduced baroresistance in mixed beverages, like fruit smoothies, when compared to PME. A PME inactivation of 83% occurred at 700 MPa/55°C evidencing resistant to pressure in a skim milk-orange juice beverage (Chakraborty, Kaushik, et al., 2014; Keenan, Rößle, Gormley, Butler, & Brunton, 2012).

The effect of the pressure on enzyme activity depends on the substrate. Moderate hydrostatic pressures (MHP) applied to mushroom increased PPO activity by 140% after treatment at 400 MPa for 10 min, when compared with the unprocessed sample (Gomes & Ledward, 1996). Carrot and apple extracts presented an increase in PPO activity after pressure treatments between 100 and 300 MPa for 1 min, which was attributed to the reversible configuration and/or conformational deviations in enzyme and/or substrate particles (Anese, Nicoli, Dall'aglio, & Lerici, 1994). The MHP applied in the range 200–500 MPa at room temperature also produced an increase in PPO activity of up to 65% in apple juice (Buckow et al., 2009).

As for the activity of PAL, the activity of PME points at some activation with increasing pressure, although the model is not significant and thus this cannot be quantified. In the experimental conditions PME's enzymatic activity ranged from 7.71 to 11.76 $U.g^{-1}$ of dry matter in pineapple shell and 7.99–11.60 $U.g^{-1}$ of dry matter in pineapple core.

PME was considered a baroresistent enzyme in two studies with pineapple puree, and stability depends on the enzyme origin (Chakraborty, Rao, & Mishra, 2014; Chakraborty, Rao, & Mishra, 2019).

The PME has shown evidence of increased activity under MHP in several studies (Eisenmenger & Reyes-De-Corcuera, 2009). Tomato juice PME was activated by pressures greater than 300 MPa (Hsu, 2008). In the case of carrot assays, PME activity in shredded samples was most evident at 50°C and 200–400 MPa and 100–400 MPa at 60°C in whole carrots (Sila et al., 2007). PME inactivation rate in carrot samples was reduced (> 75%) at 300 MPa, compared with 100 MPa (Ly-Nguyen et al., 2003). Green peppers subjected to pressure between 100 and 200 MPa showed increased activity of PME after treatment compared with untreated samples (Castro et al., 2008).

3.5 | Antioxidant activity

Regarding antioxidant activity of pineapple core samples, the obtained models present an insufficient fit to the data ($R^2 < 0.75$) and a correspondingly significant lack of fit and thus they are not presented.

Models obtained for antioxidant activity of pineapple shell samples by DPPH, FRAP and ABTS methods presented a good fit ($R^2 > 0.75$) to the data but a significant lack of fit (Table 3), so these results will be only considered as indicative of a trend and model figures are not shown.

The moderate pressures studied increase antioxidant activity 85% by DPPH method, 79% by FRAP method and 76% by ABTS method, in the pineapple shell samples (Table 2).

The second order model for the antioxidant activity in pineapple core samples does not fit the data, although there is a trend for activation of antioxidant activity with increasing pressure. The antioxidant activity of the pineapple core samples by the DPPH method ranged between 32.71–43.61 μmol $Trolox.g^{-1}$ dry matter, the FRAP method ranged between 19.16 and 36.39 μmol $Trolox.g^{-1}$ dry matter and the ABTS method ranged between 29.91 and 51.60 μmol $Trolox.g^{-1}$ dry matter.

As a conclusion, intermediate pressures allow an increase of antioxidant activity. Antioxidant activity is also influenced by treatment time, with higher values being obtained for shorter or intermediate studied treatment times.

Other authors have studied the behavior of antioxidant compounds with HPP. The application of high pressure does not reduce antioxidant compounds in the fruit sample but can increase the antioxidant capacity in pineapple (Chakraborty, Rao, & Mishra, 2015), orange, lemon and carrot mixed juice (Butz et al., 2003), and strawberry and blackberry purees (Patras, Brunton, Da Pieve, & Butler, 2009).

In another study, Sánchez-Moreno, Plaza, De Ancos, and Cano (2006) observed that, at 50–400 MPa at 25°C for 15 min, the antioxidant activity (DPPH) of the aqueous fraction of tomato puree increased. A slight increase in antioxidant activity was observed at pressures of ~200 MPa (Sánchez-Moreno, Plaza, De Ancos, & Cano, 2004).

The explanation for the increase in antioxidant activity may be the increase in extraction yields by hydrostatic pressure treatments.

Pressure may increase permeability due to the ability to deprotonate charged groups and disrupt salt bridges and hydrophobic bonds in cell membranes (Corrales, Toepfl, Butz, Knorr, & Tauscher, 2008; Raso & Barbosa-Cánovas, 2003). The higher amount of TPC and antioxidants may also be influenced by the decrease in water dielectric constant caused by hydrostatic pressure treatments associated with temperature (Fernández, Goodwin, Lemmon, Levelt Sengers, & Williams, 1997).

Polyphenol content in soluble extracts was significantly higher (17–28%) compared to other extracts. This increase may result from a rupture of plant cells caused by the treatment of moderate pressures (250–400 MPa and 3–5 min), increasing the extraction capacity of these compounds (Van Eylen, Oey, Hendrickx, & Van Loey, 2008). Hydrological pressure applied to cashew apple samples (250 MPa for 3 min) did not change FRAP measured antioxidant capacity, but the DPPH method presented a 40% increase in antioxidant capacity (Queiroz et al., 2010). The antioxidant capacity of blackberry puree increased after pressure treatment, but in strawberry puree this effect was not observed (Patras et al., 2009).

3.6 | Optimization of moderate hydrostatic pressure application conditions

The desirability function was applied for the simultaneous optimization of the responses that fitted to the second order model for pineapple shell samples (bromelain activity and phenylalanine ammonia-lyase activity) and the desirability surface curves for optimal conditions are shown in Figure 3. The curve allows observing a maximum point that corresponds to the optimal conditions obtained for the results of the models together.

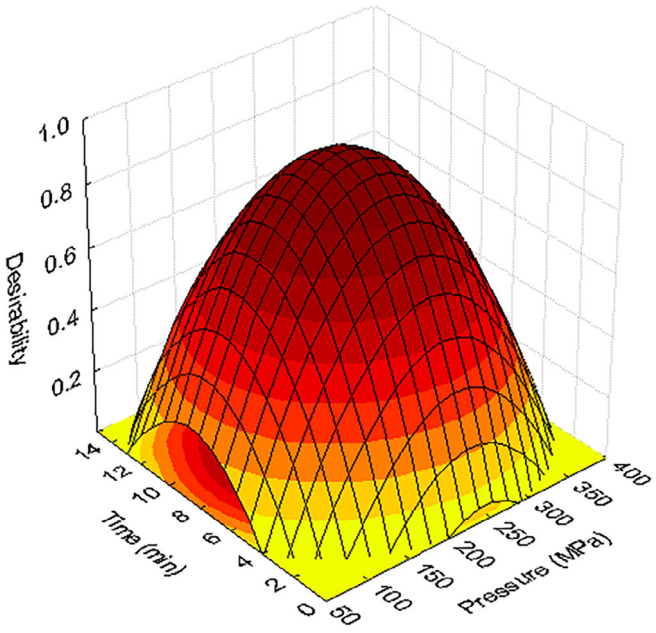

FIGURE 3 Desirability surface curves for optimal conditions as a function of pressure (MPa) and time (min) treatment in pineapple shell for bromelain activity and phenylalanine ammonia-lyase activity (PAL)

The most suitable conditions found for the MHP treatment conditions applied to pineapple shell were 225 MPa during 7.6 min, with desirability values of 0.94. Under these conditions, the predicted responses for independent studied variables are 28.61 μmol tyrosine. $min^{-1}.g^{-1}$ dry matter and 388.48 μmol t-cinnamic $acid.h^{-1}.g^{-1}$ dry matter for bromelain activity and phenylalanine ammonia-lyase activity, respectively.

In the case of the pineapple core, just the quadratic model generated for bromelain activity was significant in fitting of the experimental data. So, the optimized conditions for bromelain activity will be considered to be 211 MPa of hydrostatic pressure and 8.7 min of treatment time. At these MHP treatment conditions the predicted bromelain activity is 17.71 mg $tyrosine.min^{-1}.g^{-1}$ dry matter.

4 | CONCLUSION

The abiotic stress treatments by moderate hydrostatic pressure promote enzyme activity and induced synthesis of bioactive compounds from pineapple shell and core. The moderate hydrostatic pressure treatments (150–250 MPa) during about 8 min could activate cellular processes as a stress response, enhancing accumulation of bromelain and phenolic compounds with antioxidant activity, whereas more intense hydrostatic pressure treatments (> 300 MPa) could cause irreversible damage. The increased activity of bromelain is more evident in the pineapple core, while the increase of PAL activity is more perceptible in the pineapple shell.

The results obtained in this work showed that the interesting feature of the pineapple core is bromelain activity and in the pineapple shell it is PAL activity, and consequently TPC. In order to maximize these compounds, it is suggested to apply optimum conditions to maximize bromelain (211 MPa and 8.7 min) in the pineapple core and conditions that maximize PAL (221.5 MPa and 8.14 min) in the pineapple shell. Pineapple by-products subjected to abiotic stresses may be a good source of bioactive compounds and proteolytic enzymes (bromelain) to be incorporated directly or used in food preparation as a co-product or raw material.

This work thus contributes to valorization of agroindustry wastes, leading to economical (final product with higher added value) and environmental (reduced waste production).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

INFORMED CONSENT

Not applicable.

ORCID

Diana I. Santos https://orcid.org/0000-0001-5177-6878
Carlos A. Pinto https://orcid.org/0000-0002-5742-706X
Luiz C. Corrêa-Filho https://orcid.org/0000-0001-5405-3763
Jorge A. Saraiva https://orcid.org/0000-0002-5536-6056
António A. Vicente https://orcid.org/0000-0003-3593-8878
Margarida Moldão-Martins https://orcid.org/0000-0003-0558-8715

REFERENCES

Alegria, C., Gonçalves, E. M., Moldão-Martins, M., Cisneros-Zevallos, L., & Abreu, M. (2016). Peel removal improves quality without antioxidant loss, through wound-induced phenolic biosynthesis in shredded carrot. *Postharvest Biology and Technology*, *120*, 232–239. https://doi.org/10.1016/j.postharvbio.2016.07.004

Anese, M., Nicoli, M. C., Dall'aglio, G., & Lerici, C. R. (1994). Effect of high pressure treatments on peroxidase and polyphenoloxidase activities. *Journal of Food Biochemistry*, *18*(4), 285–293. https://doi.org/10.1111/j.1745-4514.1994.tb00503.x

Babu, B. R., Rastogi, N. K., & Raghavarao, K. S. M. S. (2008). Liquid-liquid extraction of bromelain and polyphenol oxidase using aqueous two-phase system. *Chemical Engineering and Processing: Process Intensification*, *47*(1), 83–89. https://doi.org/10.1016/j.cep.2007.08.006

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, *239*(1), 70–76. https://doi.org/10.1006/abio.1996.0292

Bhattacharyya, B. K. (2008). Bromelain: An overview. *Natural Product Radiance*, *7*(4), 359–363.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Buckow, R., Weiss, U., & Knorr, D. (2009). Inactivation kinetics of apple polyphenol oxidase in different pressure-temperature domains. *Innovative Food Science and Emerging Technologies*, *10*(4), 441–448. https://doi.org/10.1016/j.ifset.2009.05.005

Butz, P., Fernández García, A., Lindauer, R., Dieterich, S., Bognár, A., & Tauscher, B. (2003). Influence of ultra high pressure processing on fruit and vegetable products. *Journal of Food Engineering*, *56*, 233–236. https://doi.org/10.1016/S0260-8774(02)00258-3

Cano, M., & de Ancos, B. (2005). Advances in use of high pressure to processing and preservation of plant foods. *Novel Food Processing Technologies*, 283–310. Retrieved from https://www.google.com/books?hl=pt-PT&lr=&id=fpZ1Vpf17JkC&oi=fnd&pg=PA283&dq=Advances+in+use+of+high+pressure+to+processing+and+preservation+of+plant+foods&ots=1L0pbvSwLp&sig=7eqHvdPoETvGZYWdT_L2V4hkVjo

Castro, S. M., Saraiva, J. A., Lopes-da-Silva, J. A., Delgadillo, I., Van Loey, A., Smout, C., & Hendrickx, M. (2008). Effect of thermal blanching and of high pressure treatments on sweet green and red bell pepper fruits (*Capsicum annuum* L.). *Food Chemistry*, *107*(4), 1436–1449. https://doi.org/10.1016/j.foodchem.2007.09.074

Chakraborty, S., Kaushik, N., Rao, P. S., & Mishra, H. N. (2014). High-pressure inactivation of enzymes: A review on its recent applications on fruit purees and juices. *Comprehensive Reviews in Food Science and Food Safety*, *13*(4), 578–596. https://doi.org/10.1111/1541-4337.12071

Chakraborty, S., Rao, P. S., & Mishra, H. N. (2014). Effect of pH on enzyme inactivation kinetics in high-pressure processed pineapple (*Ananas comosus* L.) puree using response surface methodology. *Food and Bioprocess Technology*, *7*(12), 3629–3645. https://doi.org/10.1007/s11947-014-1380-0

Chakraborty, S., Rao, P. S., & Mishra, H. N. (2015). Effect of combined high pressure-temperature treatments on color and nutritional quality attributes of pineapple (*Ananas comosus* L.) puree. *Innovative Food Science and Emerging Technologies*, *28*, 10–21. https://doi.org/10.1016/j.ifset.2015.01.004

Chakraborty, S., Rao, P. S., & Mishra, H. N. (2016a). Changes in quality attributes during storage of high-pressure and thermally processed pineapple puree. *Food and Bioprocess Technology*, *9*(5), 768–791. https://doi.org/10.1007/s11947-015-1663-0

Chakraborty, S., Rao, P. S., & Mishra, H. N. (2016b). Modeling the inactivation kinetics of fruit bromelain in pineapple during high-pressure and thermal treatments. *Innovative Food Science and Emerging Technologies*, *33*, 10–18. https://doi.org/10.1016/j.ifset.2015.12.026

Chakraborty, S., Rao, P. S., & Mishra, H. N. (2019). Modeling the inactivation of pectin methylesterase in pineapple puree during combined high-pressure and temperature treatments. *Innovative Food Science and Emerging Technologies*, *52*, 271–281. https://doi.org/10.1016/j.ifset.2019.01.008

Chen, J. Y., Wen, P. F., Kong, W. F., Pan, Q. H., Zhan, J. C., Li, J. M., … Huang, W. D. (2006). Effect of salicylic acid on phenylpropanoids and phenylalanine ammonia-lyase in harvested grape berries. *Postharvest Biology and Technology*, *40*(1), 64–72. https://doi.org/10.1016/j.postharvbio.2005.12.017

Cisneros-Zevallos, L. (2003). The use of controlled postharvest abiotic stresses as a tool for enhancing the nutraceutical content and adding-value of fresh fruits and vegetables. *Journal of Food Science*, *68*(5), 1560–1565. https://doi.org/10.1111/j.1365-2621.2003.tb12291.x

Corrales, M., Toepfl, S., Butz, P., Knorr, D., & Tauscher, B. (2008). Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: A comparison. *Innovative Food Science and Emerging Technologies*, *9*(1), 85–91. https://doi.org/10.1016/j.ifset.2007.06.002

Corrêa-Filho, L., Lourenço, S., Duarte, D., Moldão-Martins, M., & Alves, V. (2019). Microencapsulation of tomato (*Solanum lycopersicum* L.) pomace ethanolic extract by spray drying: Optimization of process conditions. *Applied Sciences*, *9*(3), 612. https://doi.org/10.3390/app9030612

Eisenmenger, M. J., & Reyes-De-Corcuera, J. I. (2009). High pressure enhancement of enzymes: A review. *Enzyme and Microbial Technology*, *45*(5), 331–347. https://doi.org/10.1016/j.enzmictec.2009.08.001

Fernández, D. P., Goodwin, A. R. H., Lemmon, E. W., Levelt Sengers, J. M. H., & Williams, R. C. (1997). A formulation for the static permittivity of water and steam at temperatures from 238 K to 873 K at pressures up to 1200 MPa, including derivatives and Debye-Hückel coefficients. *Journal of Physical and Chemical Reference Data*, *26*(4), 1125–1166. https://doi.org/10.1063/1.555997

Gomes, M. R. A., & Ledward, D. A. (1996). Effect of high-pressure treatment on the activity of some polyphenoloxidases. *Food Chemistry*, *56*(1), 1–5. https://doi.org/10.1016/0308-8146(95)00141-7

Haaland, P. D. (1989). Experimental design in biotechnology. In D. B. Owen (Ed.), *Statistics: Textbooks and monographs* (Vol. 105, p. 259). New York, Basel: Marcel Dekker Inc.

Heredia, J. B., & Cisneros-Zevallos, L. (2009). The effect of exogenous ethylene and methyl jasmonate on pal activity, phenolic profiles and antioxidant capacity of carrots (*Daucus carota*) under different wounding intensities. *Postharvest Biology and Technology*, *51*(2), 242–249. https://doi.org/10.1016/j.postharvbio.2008.07.001

Hsu, K. C. (2008). Evaluation of processing qualities of tomato juice induced by thermal and pressure processing. *LWT - Food Science and Technology*, *41*(3), 450–459. https://doi.org/10.1016/j.lwt.2007.03.022

Jacobo-Velázquez, D. A., & Hernández-Brenes, C. (2010). Biochemical changes during the storage of high hydrostatic pressure processed avocado paste. *Journal of Food Science*, *75*(6), S264–S270. https://doi.org/10.1111/j.1750-3841.2010.01654.x

Jacobo-Velázquez, D. A., González-Agüero, M., & Cisneros-Zevallos, L. (2015). Cross-talk between signaling pathways: The link between plant secondary metabolite production and wounding stress response. *Scientific Reports*, *5*(1), 8608. https://doi.org/10.1038/srep08608

Keenan, D. F., Rößle, C., Gormley, R., Butler, F., & Brunton, N. P. (2012). Effect of high hydrostatic pressure and thermal processing on the nutritional quality and enzyme activity of fruit smoothies. *LWT - Food Science and Technology*, *45*(1), 50–57. https://doi.org/10.1016/j.lwt.2011.07.006

Ludikhuyze, L., Van Loey, A., Indrawati Smout, C., & Hendrickx, M. (2003). Effects of combined pressure and temperature on enzymes related to quality of fruits and vegetables: From kinetic information to process engineering aspects. *Critical Reviews in Food Science and Nutrition*, *43*(5), 527–586. https://doi.org/10.1080/10408690390246350

Ly-Nguyen, B., Van Loey, A. M., Smout, C., Özcan, S. E., Fachin, D., Verlent, I., … Hendrickx, M. E. (2003). Mild-heat and high-pressure inactivation of carrot pectin methylesterase: A kinetic study. *Journal of Food Science*, *68*(4), 1377–1383. https://doi.org/10.1111/j.1365-2621.2003.tb09653.x

Montgomery, D. C. (2017). *Design and analysis of experiments* (9th ed.). Hoboken, NJ, USA: John Wiley & Sons, Inc. Retrieved from https://books.google.pt/books?hl=pt-PT&lr=&id=Py7bDgAAQBAJ&oi=fnd&pg=PA1&ots=X6v_q_JS23&sig=1EU_hb8OZ5e13ldWgWIOzZXg8E4&redir_esc=y#v=onepage&q&f=false

Mozhaev, V. V., Lange, R., Kudryashova, E. V., & Balny, C. (1996). Application of high hydrostatic pressure for increasing activity and stability of enzymes. *Biotechnology and Bioengineering*, *52*(2), 320–331. https://doi.org/10.1002/(SICI)1097-0290(19961020)52:2<320::AID-BIT12>3.0.CO;2-N

Orozco-Cardenas, M. L., Narvaez-Vasquez, J., & Ryan, C. A. (2001). Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, Systemin, and methyl Jasmonate. *The Plant Cell*, *13*(1), 179–191. https://doi.org/10.2307/3871162

Patras, A., Brunton, N. P., Da Pieve, S., & Butler, F. (2009). Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innovative Food Science and Emerging Technologies*, *10*(3), 308–313. https://doi.org/10.1016/j.ifset.2008.12.004

Pavan, R., Jain, S., Shraddha, & Kumar, A. (2012). Properties and therapeutic application of bromelain: A review. *Biotechnology Research International*, *2012*, 1–6. https://doi.org/10.1155/2012/976203

Pinheiro, J., Silva, C. L. M., Alegria, C., Abreu, M., & Gonçalves, E. M. (2012). Optimization, heat stability and kinetic characterization of pectin-methylesterase enzyme from tomato ('Zinac') Fruits*solanum lycopersicum*. *Acta Horticulturae*, *934*, 1283–1290.

Queiroz, C., Moreira, C. F. F., Lavinas, F. C., Lopes, M. L. M., Fialho, E., & Valente-Mesquita, V. L. (2010). Effect of high hydrostatic pressure on phenolic compounds, ascorbic acid and antioxidant activity in cashew apple juice. *High Pressure Research*, *30*(4), 507–513. https://doi.org/10.1080/08957959.2010.530598

Raimbault, A.-K., Zuily-Fodil, Y., Soler, A., Mora, P., & Cruz de Carvalho, M. H. (2013). The expression patterns of bromelain and AcCYS1 correlate with blackheart resistance in pineapple fruits submitted to postharvest chilling stress. *Journal of Plant Physiology*, *170*(16), 1442–1446. https://doi.org/10.1016/j.jplph.2013.05.008

Raso, J., & Barbosa-Cánovas, G. V. (2003). Nonthermal preservation of foods using combined processing techniques. *Critical Reviews in Food Science and Nutrition*, *43*(3), 265–285. https://doi.org/10.1080/10408690390826527

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9–10), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Rosas, C., Domínguez, J., Pareek, S., Villegas, M., Ayala, F., Yahia, E., & González, G. (2018). Content of bioactive compounds and their contribution to antioxidant capacity during ripening of pineapple (*Ananas comosus* L.) cv. Esmeralda. *Journal of Applied Botany and Food Quality*, *91*, 61–68. https://doi.org/10.5073/JABFQ.2018.091.009

Rufino, M. S. M., Alves, R. E., Sousa De Brito, E., Maia De Morais, S., Goes Sampaio, C., Pérez-Jiménez, J., & Saura-Calixto, F. D. (2007). *Metodologia Científica: Determinação da Atividade Antioxidante Total em Frutas pela Captura do Radical Livre ABTS +*. Brazil. Retrieved from Comunicado Técnico (Embrapa Agroindústria Tropical). http://www.cnpat.embrapa.br/cnpat/down/index.php?pub/Cot_128.pdf

Sánchez-Moreno, C., Plaza, L., De Ancos, B., & Cano, M. P. (2004). Effect of combined treatments of high-pressure and natural additives on carotenoid extractability and antioxidant activity of tomato puree (Lycopersicum esculentum mill.). *European Food Research and Technology*, *219*(2), 151–160. https://doi.org/10.1007/s00217-004-0926-1

Sánchez-Moreno, C., Plaza, L., De Ancos, B., & Cano, M. P. (2006). Impact of high-pressure and traditional thermal processing of tomato purée on carotenoids, vitamin C and antioxidant activity. *Journal of the Science of Food and Agriculture*, *86*(2), 171–179. https://doi.org/10.1002/jsfa.2321

Sila, D. N., Smout, C., Satara, Y., Truong, V., Van Loey, A., & Hendrickx, M. (2007). Combined thermal and high pressure effect on carrot pectinmethylesterase stability and catalytic activity. *Journal of Food Engineering*, *78*(3), 755–764. https://doi.org/10.1016/j.jfoodeng.2005.11.016

Solecka, D., & Kacperska, A. (2003). Phenylpropanoid deficiency affects the course of plant acclimation to cold. *Physiologia Plantarum*, *119*(2), 253–262. https://doi.org/10.1034/j.1399-3054.2003.00181.x

Surjadinata, B. B., & Cisneros-Zevallos, L. (2012). Biosynthesis of phenolic antioxidants in carrot tissue increases with wounding intensity. *Food Chemistry*, *134*(2), 615–624. https://doi.org/10.1016/j.foodchem.2012.01.097

Swain, T., & Hillis, W. E. (1959). The phenolic constituents of *Prunus domestica*. I.—The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture*, *10*(1), 63–68. https://doi.org/10.1002/jsfa.2740100110

Terefe, N. S., Buckow, R., & Versteeg, C. (2014, January 4). Quality-related enzymes in fruit and vegetable products: Effects of novel food processing technologies, part 1: High-pressure processing. *Critical Reviews in Food Science and Nutrition*, *54*, 24–63. https://doi.org/10.1080/10408398.2011.566946

Tomás-Barberán, F. A., & Espín, J. C. (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *Journal of the Science of Food and Agriculture*, *81*, 853–876. https://doi.org/10.1002/jsfa.885

Van Eylen, D., Oey, I., Hendrickx, M., & Van Loey, A. (2008). Effects of pressure/temperature treatments on stability and activity of endogenous broccoli (*Brassica oleracea* L. cv. Italica) myrosinase and on cell permeability. *Journal of Food Engineering*, *89*(2), 178–186. https://doi.org/10.1016/j.jfoodeng.2008.04.016

Zarei, A., Zamani, Z., Fattahi, R., Salami, A., & Mousavi, A. (2016). Analysis of the phenylpropanoid enzyme activities and products in the soft- and hard-seeded pomegranate genotypes during fruit development. *International Journal of Fruit Science*, *16*(3), 242–258. https://doi.org/10.1080/15538362.2015.1089814

Zhou, Y., Dahler, J. M., Underhill, S. J., & Wills, R. B. (2003). Enzymes associated with blackheart development in pineapple fruit. *Food Chemistry*, *80*(4), 565–572. https://doi.org/10.1016/S0308-8146(02)00375-8

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